

Playing Darwin. Part B. 20 years of domestication in *Drosophila subobscura*

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Received: 25 June 2009 / Accepted: 2 November 2009 / Published online: 8 June 2010
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Abstract Adaptation to a new environment (as well as its underlying mechanisms) is one of the most important topics in Evolutionary Biology. Understanding the adaptive process of natural populations to captivity is essential not only in general evolutionary studies but also in conservation programmes. Since 1990, the Group of Experimental Evolution (CBA/FCUL) has been performing long-term, real-time evolutionary studies, with the characterization of laboratory adaptation in populations of *Drosophila subobscura* founded in different times and from different locations. Initially, these experiments involved phenotypic assays and more recently were expanded to studies at the molecular level (microsatellite and chromosomal polymorphisms) and with different population sizes. Throughout these two decades, a clear pattern of evolutionary convergence to long-established laboratory populations has been consistently observed in several life-history traits. However, contingencies across foundations were also found during the adaptive process. In characters with complex evolutionary trajectories, the data suggested that

the comparative method lacked predictive capacity relative to real-time evolutionary trajectories (experimental evolution). Microsatellite analysis revealed general similarity in gene diversity and allele number between studied populations, as well as an unclear association between genetic variability and evolutionary potential. Nevertheless, ongoing studies in all foundations are being carried out to further test this hypothesis. A comparison between recently introduced and long-term populations (founded from the same natural location) has shown higher degree of chromosomal polymorphism in recent ones. Finally, our findings suggest higher heterogeneity between small-sized populations, as well as a slower evolutionary rate in characters close to fitness (such as fecundity and mating behaviour). This comprehensive study is aimed at better understanding the processes and patterns underlying adaptation to captivity, as well as its genetic basis.

Keywords Domestication · *Drosophila subobscura* · Adaptation · Experimental evolution

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Introduction

“From a remote period, in all parts of the world, man has subjected many animals and plants to domestication or culture. Man has no power of altering the absolute conditions of life; he cannot change the climate of any country; he adds no new element to the soil; but he can remove an animal or plant from one climate or soil to another, and give it food on which it did not subsist in its natural state.” (Darwin 1868).

Domestication is historically one of the most important topics in Evolutionary Biology and may be considered the most ancient evolutionary experiment made by humans.

Although the events have occurred at different times and places, the first records on domestication date back to 14000 years ago; predominantly in the Middle East and Asia (Fertile Crescent). Dogs and various species of livestock were the first animals to be domesticated (Mignon-Grasteau et al. 2005). Plant species were also domesticated by humans, who were responsible for their outward dispersal (Diamond and Bellwood 2003). Traditionally, domestication refers to the genetic changes undergone by our commensal species (from wolves to dogs, from einkorn to wheat), but a more useful definition for scientific purposes is that domestication is the evolutionary genetic change arising from the transition of a population from nature to deliberate human cultivation (Simões et al. 2007).

“When a species or population cannot avoid an increasingly stressful environment by shifting its geographic distribution, its adaptation or extinction will be determined decisively by the ability to mobilize sufficient genetic variation to track the environmental change” (Lande and Shannon 1996; Rodríguez-Trelles et al. 1998). Ultimately, populations may become threatened because they are not able to respond to changes in the habitat imposed by man, and they may need to be maintained in captivity until the habitat is recovered (Frankham et al. 2002). Therefore, understanding the adaptive process of natural populations to captivity (study of evolutionary rates, population differentiation, etc.) is essential not only in general evolutionary studies but also in conservation programmes (Simões et al. 2007, 2009). Particularly in ex situ conservation programmes, evolutionary changes throughout the process of adaptation to captivity may seriously compromise the success of reintroducing populations to the wild (Frankham 1995; Frankham et al. 2000, 2002; Gilligan and Frankham 2003). Characterizing the evolutionary dynamics during adaptation to captivity in threatened species is not a viable project. Instead several teams have turned to model organisms like *Drosophila*, used as surrogate organisms to highlight the evolutionary consequences of prolonged ex situ conservation programmes (Frankham 2005a).

Since 1990, the Group of Experimental Evolution (CBA/FCUL) has been performing long-term real-time evolutionary studies in laboratory populations of *Drosophila subobscura*, founded in different times and locations. Initially, these experiments involved phenotypic assays and more recently were expanded to the molecular level (microsatellite and chromosomal polymorphisms) and different population sizes. Here we will give a brief highlight of some of the results obtained in our studies with the intention of illustrating the potentialities of this type of research programme both for evolutionary and conservation biology.

Materials and methods

All laboratory populations (Fig. 1) derive from natural populations of *Drosophila subobscura* from two different locations (Sintra and Arrábida, Portugal). The first laboratory population (NB) was founded in 1990 (from Sintra) and has been used as a control since 1998. Other foundations were subsequently made in 1998, 2001, 2005, 2007 and 2008. In 2001 and 2005 foundations from Arrábida and Sintra were collected synchronously. The foundations of 2005 consisted of two independent collections from each of the two natural sites in order to test for sampling effects on the early adaptive dynamics (one of the two foundations from each location being extinguished after 21 generations—see Fig. 1). All populations were three-fold replicated at their second generation in the laboratory (except for NB which was replicated only at its 90th generation)—see Matos et al. (2000, 2004) and Simões et al. (2007, 2008a).

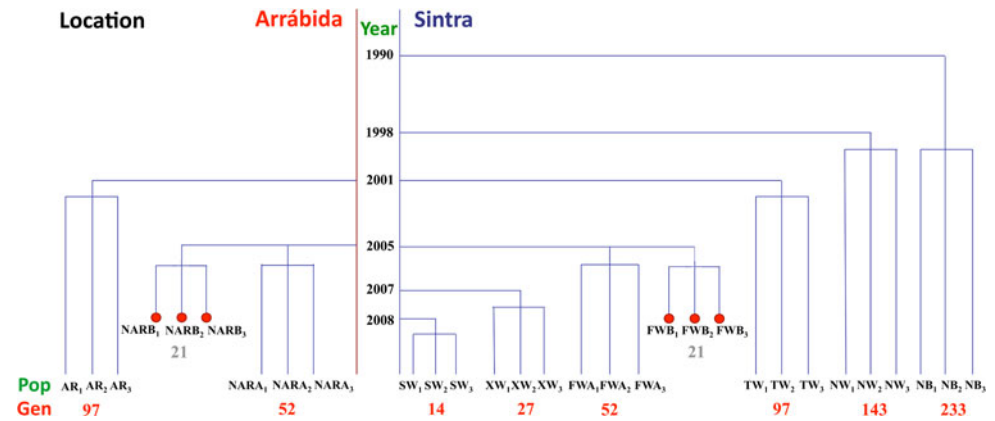
The laboratory populations were maintained under the same conditions, involving discrete generations of 28 days, reproduction close to peak fecundity and controlled temperature of 18°C with a 12 h-light:12 h-dark photoperiod. Flies were kept in vials, with controlled adult densities (around 50 individuals per vial) and larval densities (80 per vial). At each generation, emergences from the several vials within each population were randomized using CO₂ anaesthesia. Adult population sizes ranged in general from 600 to 1200 individuals, except for the small-sized populations (PW, derived from XW at their 3rd generation), which had about 50 individuals.

The life-history traits assays were performed as described by Simões et al. (2008a). Microsatellite genotyping and chromosomal inversions involved the procedures described by Simões et al. (2008b) and Balanyà et al. (2004).

Experimental evolution in *Drosophila subobscura*: real-time evolutionary studies

It is a basic corollary of evolutionary theory that populations exposed to a novel environment are expected to undergo adaptation to that environment. Such adaptation can be defined as an increase of the mean fitness in the new environment due to genetic change. Since the laboratory is just another environment in the evolutionary history of a population, where a general pattern of adaptation might be expected (Matos et al. 2000), a temporal increase in fitness and its related traits (e.g. early fecundity) is predicted. Overall our data have shown a clear and repeatable pattern of convergence to the control in all the laboratory populations, independently of year and/or location of foundation

Fig. 1 “Phylogeny” of all laboratory populations, indicating the original wild location and year of foundation. Generation number for each set of populations corresponds to their respective number of generations in the laboratory by April 2009. Besides these populations we have studied another set, PW, derived from XW at generation 3, maintained at smaller population size (see also the “Material and methods” section)



(Fig. 2). In the short-term analysis of the adaptive dynamics (Fig. 2a) our results revealed an evolutionary trajectory with a positive linear trend, whereas the long-term outcome (Fig. 2b) displayed a logarithmic tendency. These results are congruent with an initial high selective pressure and sharp evolutionary rate, followed by its progressive decrease, due to the gradual exhaustion of genetic variance of fitness (see also Matos et al. 2002, 2004; Simões et al. 2007, 2008a). In spite of the common pattern

of convergence, we have also observed that the initial differentiation (data not shown) and the early evolutionary rate present contingencies due to year (e.g. Early fecundity—Fig. 3a) and/or location of foundation (e.g. Peak fecundity—Fig. 3b)—see also Simões et al. (2008a).

Alongside the phenotypic approach, molecular analysis is a useful tool to better understand the adaptive process. The microsatellite analysis of an early generation after foundation revealed general similarity in gene diversity (H_E) and allele number (n_A) in the 2001 foundations (TW and AR, see Fig. 4). Also the evolutionary dynamics of these populations has revealed a slow temporal decrease in

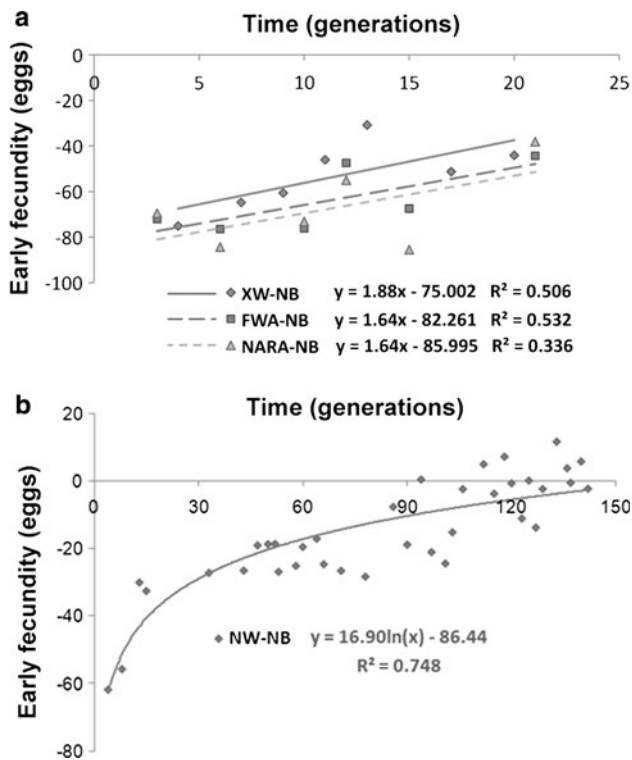


Fig. 2 Evolutionary trajectories for early fecundity. All trajectories were drawn using the average differences between replicate populations and their respective controls. The regression model with the best fit is presented. **a** Short-term studies for XW, FWA and NARA (first 20–21 generations); **b** Long-term study for NW (142 generations)

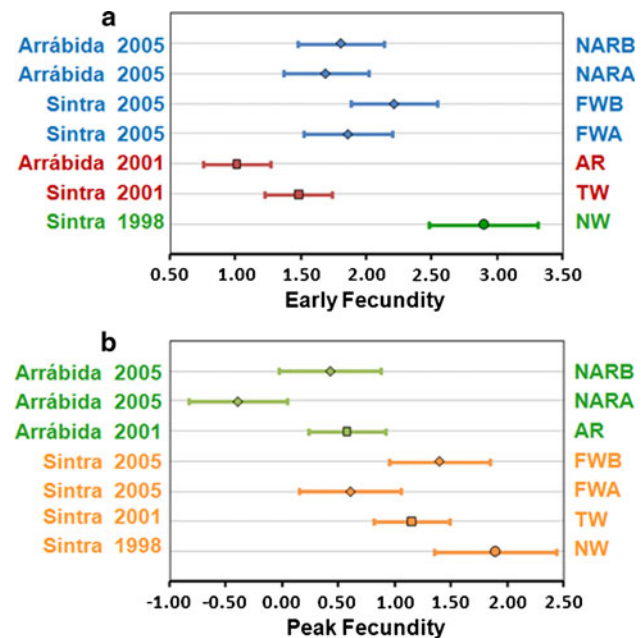


Fig. 3 Early evolutionary rate for the populations founded between 1998 and 2005. For each set of populations the average slope for the first 15–21 generations after foundation is presented (linear regression of the differences to control). **a** Early fecundity; **b** Peak fecundity. For each trait and foundation the bar represents the standard error of the slope estimated from the linear mixed-effects model: trait ~ generation + foundation + generation * foundation

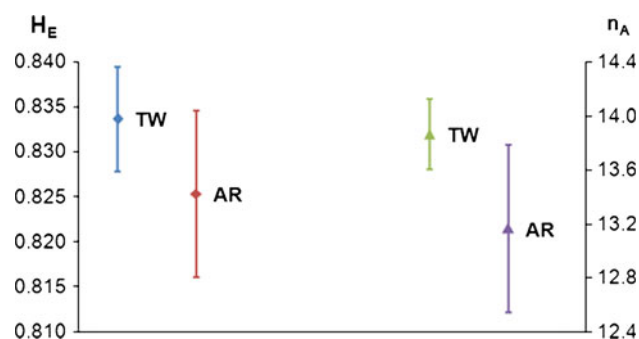


Fig. 4 Gene diversity (H_E) and allele number (n_A) for TW and AR populations at generation 3. The average values are presented (H_E on the left and n_A on the right). Vertical bars: $1.96 \times$ standard error

gene diversity, associated with an effective population size of around 20% of the census size (Simões et al. 2008b). One important issue in conservation genetics is the association between genetic variability in genetic markers and evolutionary potential (Frankham 2005a). Although the 2001 data suggest such an association (with TW showing faster evolution and higher genetic variability than AR, though not significantly so), studies involving the other foundations will allow us to further test this hypothesis (analysis in progress).

Finally, recent findings have shown that recently introduced populations (SW) presented a higher chromosomal polymorphism than long-established populations (TW). More analyses are being done in order to understand the evolutionary mechanisms—e.g. selection or genetic drift—involved in the differential loss or fixation of chromosomal inversions during laboratory adaptation. The cross of this information with analysis of molecular markers and gene expression will allow us to survey candidate genes involved in laboratory adaptation (ongoing project).

Comparative method versus experimental evolution

Several studies have used a comparative method to infer the evolutionary patterns in laboratory adaptation (e.g. Sgrò and Partridge 2000; Hoffmann et al. 2001) as opposed to the analysis of evolutionary trajectories in real-time studies, as done in our projects (described above). By comparative method we mean the inference of the evolutionary dynamics of a population based on comparisons amongst several contemporaneous populations, at different stages of evolution. Such approach assumes that each population will accurately reflect the evolutionary state of a given population at a particular moment (see Simões et al. 2009; see also Matos, this volume). This assumption can be clearly violated, e.g. due to changes in the natural population from which the several laboratory populations

derive. The question is: how much do evolutionary patterns inferred by such a method correspond to real evolutionary trajectories?

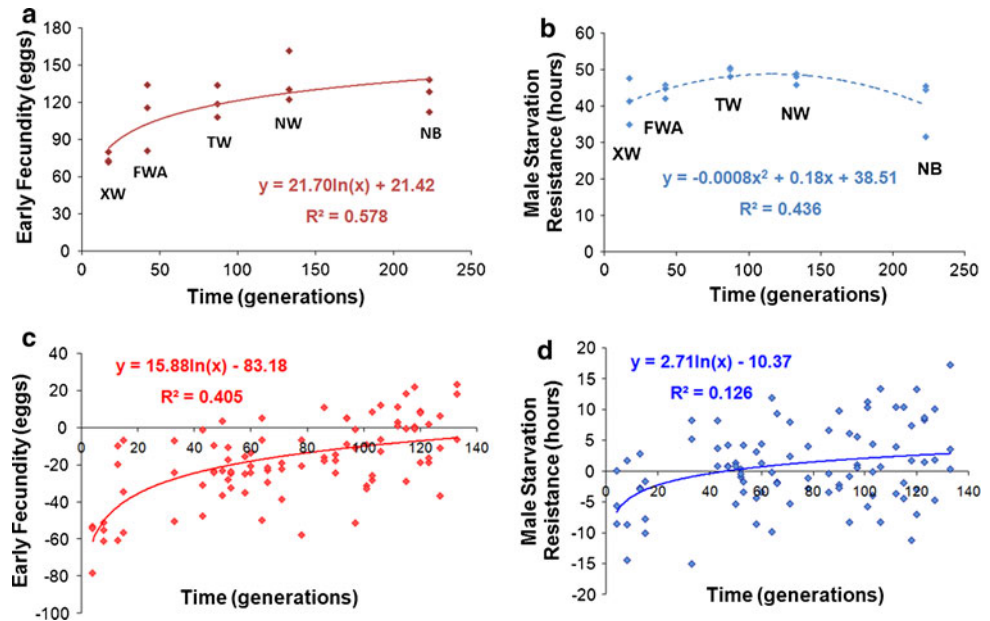
Given that we have in our laboratory several sets of populations derived from different foundations we can measure the disparity between these two approaches. Our results for early fecundity and male starvation resistance using the two approaches are shown in Fig. 5. For early fecundity there are significant log-linear regressions both for the evolutionary trajectory (Fig. 5c, $P < 0.0001$) and the comparative plot (Fig. 5a, $P < 0.002$). Moreover the two models are not significantly different (ANCOVA test of homogeneity of slopes, $P > 0.266$). In contrast, the evolutionary trajectory for male starvation resistance has a significant fit to a log-linear regression (Fig. 5d, $P < 0.001$) while the comparative plot for this trait does not (Fig. 5d, $P > 0.634$). Curiously, there is a significant fit of the last data to a 2nd degree polynomial ($P < 0.04$). These results show that the comparative method may lead to qualitatively similar evolutionary patterns as an analysis of actual evolutionary trajectories in direct fitness-related traits (such as early fecundity). Nevertheless, in characters with complex evolutionary trajectories, such as starvation resistance, the comparative method lacks predictive capacity (see also Matos et al. 2004, Simões et al. 2009).

Population size, genetic drift and conservation implications

The outcome of evolution is a balance between the deterministic effects of natural selection and the stochastic effects of genetic drift. Since genetic drift is more significant in smaller populations, evolutionary dynamics during adaptation to captivity will be affected by population size as it determines the relative importance of the two mechanisms. Conversely, bigger populations are also expected to respond faster when adapting to a novel environment (Hartl and Clark 2007; Woodworth et al. 2002). Thus, population size affects selection and genetic drift in reverse directions, shaping the evolutionary dynamics of a population during the process of adaptation. Moreover, if two different populations are kept in the same selective conditions, genetic drift will cause divergence between them opposing the expected more uniform effect of natural selection. Finally, population size may affect the likelihood of extinction of captive populations both by accumulation of mutations and demographic stochastic events (Frankham et al. 2002; Frankham 2005b).

Considering the above, understanding the effect of population size on adaptation to captivity is essential both in general evolutionary terms and to devise means to minimize the deleterious consequences upon reintroduction

Fig. 5 Comparative method versus experimental evolution. **a, b** Comparative plots for early fecundity and male starvation resistance (respectively) of independently founded populations as a function of the number of generations in the laboratory. **c, d** Evolutionary trajectories of NW populations for early fecundity and male starvation resistance (respectively); data refers to the average differences between replicate populations and their respective controls. The regression models with the best fit are presented



in the natural habitat. In fact, the best population size in ex situ management programmes has been under debate (Woodworth et al. 2002).

Our studies of laboratory adaptation in populations of contrasting sizes (50 vs. 1200 individuals) corroborate theoretical predictions. Smaller populations presented a higher heterogeneity in each assayed generation as well as a slower adaptive dynamics in characters close to fitness (such as early fecundity—see Fig. 6).

The extent to which the slower adaptive dynamics of smaller populations implicates a higher performance in the wild remains to be studied, a scenario that may occur if the best adapted in captivity is the worst in natural environment (genetic trade-off across environments).

Final remarks

The study of laboratory adaptation contributes to our understanding of evolutionary dynamics of local adaptation in general as well as adaptation to captivity in particular. Our long-term studies illustrate the ubiquity of adaptation, with several independent foundations improving their performance throughout the generations in the laboratorial (novel) environment. Adaptation is thus a universal phenomenon. Nevertheless we have found contingencies associated with effects of foundation (Simões et al. 2007, 2008a), which show that evolution is also local (Rose et al. 2005). The unclear association between initial genetic variability in neutral markers and the evolutionary potential of populations further limits predictions of their capacity to adapt to environmental changes. Ongoing studies covering all foundations will highlight this issue.

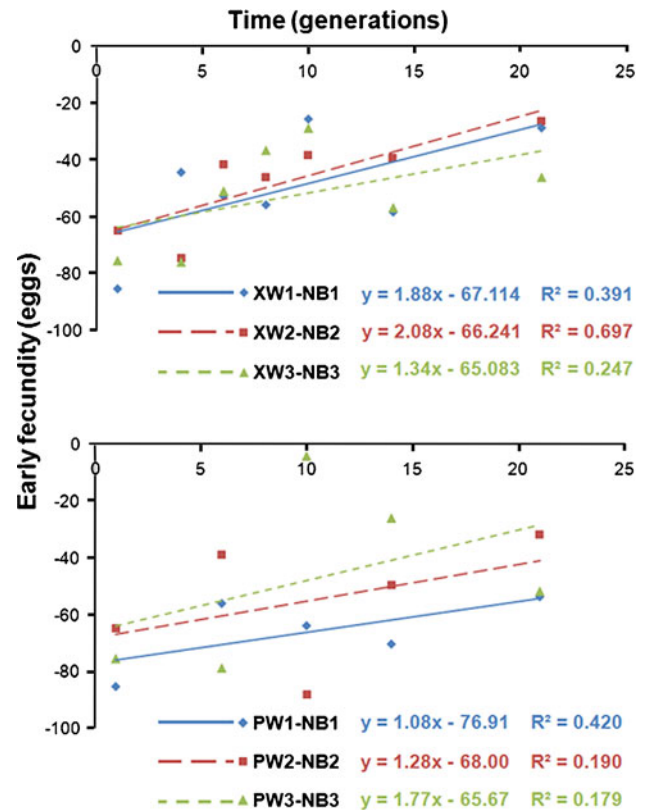


Fig. 6 Evolutionary trajectories of early fecundity for XW (large size) and PW (small size) populations. The first 21 generations after the derivation of PW are shown. All trajectories were drawn using the difference between each replicate population and its respective control. The regression model with the best fit is presented

These results show the dangers of extrapolating conclusions between organisms, as well as environmental captive conditions, in the definition of general guidelines in

conservation programmes. Moreover, we are far from knowing whether genetic trade-offs between captive and natural environments are present, in which case adaptation to captivity may threaten the success of reintroducing populations to their natural environment.

More studies involving all these issues are urgent. Meanwhile, the best strategy will be to avoid prolonged maintenance of natural populations in captivity as much as possible.

Acknowledgements The authors wish to thank Michael R. Rose and Marta Pascual for their collaboration throughout the development of the projects. This study was financed by “Fundação para a Ciência e Tecnologia” (FCT) project n° POCTI/BSE/33673/2000, by FCT and POCI 2010 project n° POCI-PPCDT/BIA-BDE/55853/2004 (both with co-participation of FEDER) and by FCT n° PTDC/BIA-BDE/65733/2006. M.S. has a PhD grant (SFRH/BD/46363/2008), J.S. has a PhD grant (SFRH/BD/28498/2006), P.S. had a PhD grant (SFRH/BD/10604/2002) and I.F. and A.M. have BTI grants from FCT.

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References

- Balanyà J, Sole E, Oller JM, Sperlich D, Serra L (2004) Long-term changes in the chromosomal inversion polymorphism of *Drosophila subobscura*. II. European populations. *J Zool Syst Evol Res* 42:191–201
- Darwin CR (1868) The variation of animals and plants under domestication, vol 1, 1st edn. John Murray, London
- Diamond J, Bellwood P (2003) Farmers and their languages: the first expansions. *Science* 300:597–603
- Frankham R (1995) Conservation genetics. *Annu Rev Genet* 29:305–327
- Frankham R (2005a) Stress and adaptation in conservation genetics. *J Evol Biol* 18:750–755
- Frankham R (2005b) Genetics and extinction. *Biol Conserv* 126:131–140
- Frankham R, Manning H, Margan SH, Briscoe DA (2000) Does equalization of family sizes reduce genetic adaptation to captivity? *Animal Conserv* 3:357–363
- Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge
- Gilligan D, Frankham R (2003) Dynamics of genetic adaptation to captivity. *Conserv Genet* 4:189–197
- Hartl DL, Clark AG (2007) Principles of population genetics, 4th edn. Sinauer Associates, Sunderland
- Hoffmann AA, Hallas R, Sinclair C, Partridge L (2001) Rapid loss of stress resistance in *Drosophila melanogaster* under adaptation to laboratory culture. *Evolution* 55:436–438
- Lande R, Shannon S (1996) The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* 50:434–437
- Matos M, Rose MR, Rocha Pitê MT, Rego C, Avelar T (2000) Adaptation to the laboratory environment in *Drosophila subobscura*. *J Evol Biol* 13:9–19
- Matos M, Avelar T, Rose MR (2002) Variation in the rate of convergent evolution: adaptation to a laboratory environment in *Drosophila subobscura*. *J Evol Biol* 15:673–682
- Matos M, Simões P, Duarte A, Rego C, Avelar T, Rose MR (2004) Convergence to a novel environment—comparative method versus experimental evolution. *Evolution* 58:1503–1510
- Mignon-Grasteau S, Boissy A, Bouix J, Faure JM, Fisher AD, Hinch GN, Jensen P, Neindre P, Mormède P, Prunet P, Vandeputte M, Beaumont C (2005) Genetics of adaptation and domestication in livestock. *Livest Prod Sci* 93:3–14
- Rodríguez-Trelles F, Rodríguez M, Scheiner S (1998) Tracking the genetic effects of global warming: *Drosophila* and other model systems. *Conserv Ecol* 2: 2. Available via <http://www.consecol.org/vol2/iss2/art2/> Accessed 12 June 2009
- Rose MR, Passananti HB, Chippindale AK, Phelan JP, Matos M, Teotónio H, Mueller LD (2005) The effects of evolution are local: evidence from experimental evolution in *Drosophila*. *Integr Comp Biol* 45:486–491
- Sgrò CM, Partridge L (2000) Evolutionary responses of the life history of wildcaught *Drosophila melanogaster* to two standard methods of laboratory culture. *Am Nat* 156:341–353
- Simões P, Rose MR, Duarte A, Gonçalves R, Matos M (2007) Evolutionary domestication in *Drosophila subobscura*. *J Evol Biol* 20:758–766
- Simões P, Santos J, Fragata I, Mueller LD, Rose MR, Matos M (2008a) How repeatable is adaptive evolution? The role of geographical origin and founder effects in laboratory adaptation. *Evolution* 62:1817–1829
- Simões P, Pascual M, Santos J, Rose MR, Matos M (2008b) Evolutionary dynamics of molecular markers during local adaptation: a case study in *Drosophila subobscura*. *BMC Evol Biol* 8:66
- Simões P, Santos J, Matos M (2009) Experimental evolutionary domestication. In: Garland T, Rose MR (eds) Experimental evolution: concepts, methods, and applications of selection experiments. California University Press, Berkeley, CA, pp 89–110
- Woodworth L, Montgomery M, Briscoe D, Frankham R (2002) Rapid genetic deterioration in captive populations: causes and conservation implications. *Conserv Genet* 3:277–288